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A Cytotoxic Flavanone from The Pod Peels of *Theprosia vogelii* Hook.f.

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Abstract

Tephrosia vogelii Hook.f. is a species of the family Fabaceae (Leguminoceae). These plants are termed ″Polong-polongan″ in Indonesia, and are known to contain active flavonoid groups. Previous studies have shown the isolation of one known flavanone: isolonchocarpin from methanol extract, and the structure obtained was established based on chemical evidence as well as spectroscopic methods, including NMR, and also by a comparison with published data. This research is aimed at evaluating the cytotoxic property of methanol extract against larvae of *Arthemia salina* Leach, using the Brine Shrimp Lethality Test (BSLT) method. The results show potent cytotoxicity at LC_{50} of 41.40 ppm.

Keyword: *Arthemia salina* Leach., cytotoxic activity, Fabaceae, flavanone, *Tephrosia vogelii* Hook.f.

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1. INTRODUCTION

Tephrosia is one of the 400 species in family Fabaceae, which belongs to the sub-family Papilionoideae, comprising shrubs, herbs and trees (Polhill, *et al*., 1981), distributed across the tropics and sub-tropics (Tarus, *et al*., 2002). Previous studies on the intrinsic chemical constituents showed the occurrence of chalcones (Andrei, *et al*., 2000; Chang, *et al*., 2000; Gomez-Garibay, *et al*., 2002; Tarus, *et al*., 2002), flavanones (Chang, *et al*., 2000; Jang, *et al*., 2003; Kishore, *et al*., 2003), flavones (Prabhakar, *et al*., 1996; Achmad, *et al*., 2007), isoflavones (Yenesew, *et al*., 1989; Dagne, *et al*., 1992; Chang, *et al*., 2000), rotenoid (Prashant, *et al*., 1993; Andrei, *et al*., 1997; Jang, *et al*., 2003). Table 1 and Figure 1 demonstrate several highly diverse phytochemical compounds present, as generally observed in most other similar genera, including Cassia from Fabaceae (Kusumaningtyas, *et al*., 2020).

The phytochemical and pharmacological evaluations showed cytotoxic potentials in several compounds (Roy, *et al*., 1986; Ganapaty, *et al*., 2009). Touqeer, *et al*. (2013) performed similar research and reported various activities from several isolates, including cytotoxicity, as observed in Table 2 (Padmapriya, 2017). This current investigation evaluates the phytochemicals and bioactivities of compounds obtained from Indonesian Tephrosia. The results showed the cytotoxic effects of pod peel methanol extract from *T. vogelii* against the *Arthemia salina* Leach larvae, with a Brine Shrimp Lethality Test (BSLT) method with LC_{50} of 41.40 ppm.

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Table 1. Tephrosia chemical constituents

Chemical constituents	Plant	References
Chalcones: tefron **(1)**	*T. candida* (seed)	Tanaka, *et al*., 1992
Flavanones: maksimaflavanon A **(2)**	*T. maxima* (root)	Rao, *et al*., 1994
Flavones: fulvinervin B **(3)**	*T. fulvinervis* (seed)	Rao, *et al*., 1985
Isoflavones: kalopogoniumisoflavon B **(4)**	*T. maxima* (root)	Murthy, *et al*., 1985
Rotenoid: 9-demetildihidrostemonal **(5)**	*T. pentaphylla* (root)	Dagne, *et al*., 1989

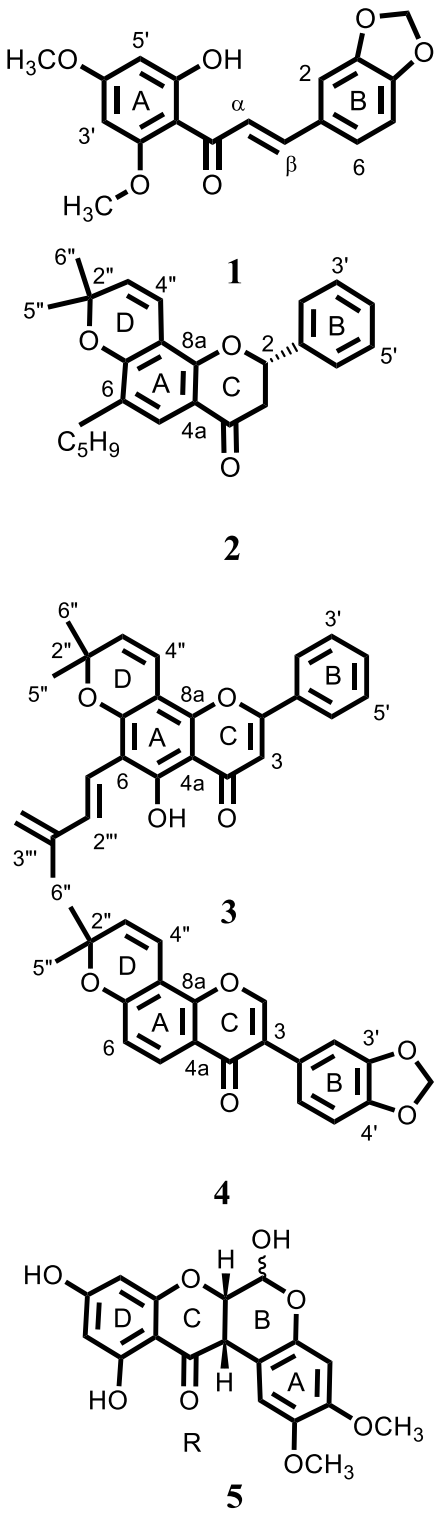

Figure 1. Structures of chemical constituents in Tephrosia

Table 2. Cytotoxic activity of *Tephrosia purpurea* extracts

Extracts	Cytotoxicity (IC_{50} value µg/mL)
Leaves	$95.73 \pm 9.60^{*\#\S}$
Root	$382.33 \pm 18.78^{\S}$
Stem	324.80 ± 21.20
Seed	303.97 ± 24.31

Mean ± SD, n = 3. $P < 0.05$ significantly different when compared to root*, stem# and seed§. (Padmapriya, *et al*., 2017)

2. MATERIALS AND METHODS

Experimental Procedure

The melting points were obtained using a micro Fisher-John, while the NMR data were recorded with JEOL ECA 500 (^{1}H 500 MHz; ^{13}C 125 MHz), where tetramethylsilane served as an internal standard. In addition, mass spectra were obtained on LCT XE ESI-TOF waters, and Centrifugal Thin-Layer Chromatography (Chromatotron) was performed on the silica gel 200 mesh using a precoated silica gel 60 GF_{254} (0.25 mm thickness) with various mobile phases. Subsequently, the spots were visualised by 1.5% cerium sulfate in 2 N sulfuric acid spaying agent, followed by heating. The NMR spectrum was then measured using residual peaks and deuterated solvents $CDCl_3$.

Plant Material

The *T. vogelii* pod peels were obtained at the Plant Taxonomy Laboratory, Biology Department, FMIPA, Unpad, West Java Province, Indonesia in March 2016. These samples were identified by Drs. Joko Kusmoro, M.P. at the Jatinangor Herbarium, Indonesia, and were deposited at the herbarium with number 183/HB/03/2016.

Brine Shrimp Lethality Assay

The bioassay indicator used to monitor plant extract toxicity during fractionation involved larva *A. salina*. Moreover,

propyleneglycol/Tween 80/water (4:1:4) in 5 mL of saltwater was applied as a negative control, while ten milligrams of potassium dichromate dissolved in prophyleneglycol/Tween 80/water (4:1:4) served as the positive control. The assessment procedure was as follows: ten shrimps were transferred to each sample vial containing extracts at varied concentrations of 500, 250, 125, 62.5, 31.2, 15.6, 7.8, and 0 ppm (control). Therefore artificial seawater for hatched Brine shrimp (*A. salina*) eggs, prepared from commercial sea salt 38 g/L (Sasidharan, *et al*., 2008) was added to each sample vial to make 5 mL. This was followed by test tubes examination, and the number of dead larvae in each bottle was counted after 24 hours. The respective death percentage were determined and the test was performed triplicate. Consequently, statistical analysis used for determining the death percentage (Equation 1) and lethal concentration (LC_{50}). (Sahgal, *et al*., 2010).

$$\%PD = \frac{(Tn-An)}{(Tn)} \times 100\% \quad \text{.....(1)}$$

Where PD is percentage of death, Tn is total nauplii and An is alive nauplii. In addition, probit analysis was performed after obtaining mortality rate. This evaluation was conducted to calculate the LC_{50}, defined as the concentration required for a compound to produced 50% death. Subsequently, the results were counted by using linear regression equation y = a + bx, and the statistical analysis (Melina, *et al*., 2020) were expressed as the mean value ± standard error of mean (SEM), while the variance was evaluated through ANOVA test. The extract toxicity level was organized according to Meyer (1982) classification. In addition, LC_{50} scores in the range ≤ 30 ppm were defined as highly toxic, while ≤ 1000 ppm were attributed as toxic, and ≥ 1000 ppm was not toxic.

Extraction and Isolation

The powdered and dried pod peels of *T.vogelii* (35 g) were extracted to yield a crude methanol extract (18.2 g). These products were then subjected to vacuum liquid chromatography over silica gel 60 GF254, and was eluted with *n*-hexane-EtOAc (10:0–0:10) in a polarity gradient manner (Syah, *et al*., 2006), and a total of fourteen (I-XIV) fractions were produced. Subsequently, fraction I was purified through centrifugal chromatography and eluted with chloroform-EtOAc (8:2) to generate isolonchocarpin (80 mg), and the structures were elucidated based on NMR data, and also through comparison with reported spectra values (Athipornchai, *et al*., 2008).

3. RESULTS AND DISCUSSION

The isolates were obtained as a pure colourless and transparent compounds in the form of needle crystals, with a melting point of 114-115 °C. Table 3 shows the NMR spectrum, featuring the signals of two proton *double doublets* at 2.84 δ_H and 3.00 ppm, respectively assigned to the H3α and H3β. Also, one proton signal *double doublets* was observed at δ_H 5.47 ppm for H-2, and two emerged from the two shielding carbons at δ_C 79.8 and 44.4 ppm for (C-2 and C-3). These were further determined as characteristic for flavanone group compounds. The nature of this structure was also confirmed by the presence of a carbon signal C = O ketone conjugated at δ_C 190.6 ppm (Athipornchai, 2008). In addition, the ^{13}C NMR spectrum also demonstrated eighteen signals for twenty carbon atoms, including for one ring unit dimethyl tetrahydropyran (δ_C 116.0; 129.0; 77.6; 28.5; 28.2 ppm), and two oxyaryl (δ_C 159.7; 157.7 ppm). Table 3 shows the ^1H NMR spectrum, indicating the presence of an aromatic signal for five protons at δ_H 7.48; 7.43 and 7.39 ppm, corresponding to the unsubstituted B ring. The two oxygen functional groups are further confirmed to occur in ring A, located at C-8a and C-7, according to the prevalence of the oxygen pattern. This phenomenon facilitates the formation of dimethyl tetrahydropyran ring at C-8 or C-6. In addition, the presence of an ortho-coupled aromatic as a *doublet* proton signal (J = 8.6 Hz) at δ_H 7.75 and 6.51 ppm respectively denote chemical shifts initiated at C-8. The HMBC spectrum shows the multiplicities and the weak coupling constants between the signal from the aromatic doublet proton at δ_H 7.75 ppm (H-5) and a C = O ketone conjugated carbon at δ_C 190.6 ppm (C-4), Also, a correlated was established between the aromatic double protons at δ_H 6.51 ppm (H-6) with oxyaryl carbon signals at δ_C 159.7 ppm (C-7), alongside two quaternary carbons at δ_C 109.5 and 114.8 ppm, respectively situated by C-8 and C-4a. According to the

coupling constant in H-2 / H-3, the configurations at C-2 and C-3 were determined to be *trans* (J = 13.2 Hz), with absolute stereochemistry assumed to follow the prevalence of 2S, 3R-flavanone. Table 3 shows the evidence of this outcome, indicated by the coupling constant in H-2 / H-3, as (J H-2 / H-3β = 13.2 Hz), (J H-2 / H-3α = 3.0 Hz) and (J_{gem} H-3α / H-3β = 16.8 Hz). Based on the spectroscopic data comparison with previous research conducted Athipornchai, *et al*. (2008), the compound is identified as isolonchocarpin.

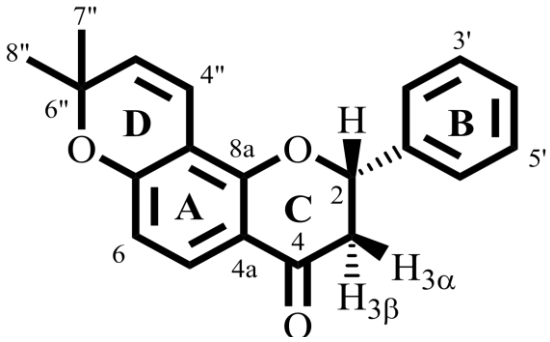

Figure 2. Chemical structure of isolonchocarpin

Table 3. NMR data for isolonchocarpin

Position	δ_H (mult., J Hz) ppm	δ_C (ppm)		HMBC (^{1}H ⇔ ^{13}C)	
	isolonchocarpin	isolonchocarpin	isolonchocarpin*	^2J	^3J
2	5.47 (*dd*, 3.0, 13.2)	79.8	79.8	2'	4
3β	3.00 (*dd*, 13.2, 16.9)	44.4	44.4	2, 4	1'
3α	2.84 (*dd*, 3.0, 16.8)	44.4	44.4	4	4a
4	-	190.6	190.6	-	-
4a	-	114.8	114.8	-	-
5	7.75 (*d*, 8.6)	127.9	127.9	-	4
6	6.51 (*d*, 8.6)	111.3	111.3	8, 4a	-
7	-	159.7	159.7	-	-
8	-	109.5	109.5	-	-
8a	-	157.7	157.7	-	-
1′	-	139.1	139.0	-	-
2′	7.48 (*dd*, 2.0, 7.9)	126.1	126.0	3′	2, 6′
3′	7.43(*dd*, 7.9, 8.2)	128.9	128.8	-	5′
4′	7.39 (*dt*, 2.0, 8.2)	128.7	128.6	3′, 5′	-
5′	7.43(*dd*, 7.9, 8.2)	128.9	128.8	-	-
6′	7.48 (*dd*, 2.0, 7.9)	126.1	126.0	5′	2′
4″	6.66 (*d*, 10.1)	116.0	115.9	8	7, 8a
5″	5.57 (*d*, 10.1)	129.0	128.9	6″	8
6″	-	77.6	77.6	-	-
7″-CH_3	1.47 (*s*)	28.2	28.1		5″, 8″
8″-CH_3	1.45 (*s*)	28.5	28.4	6″	-

*Athipornchai *et al*. (2008); ^{1}H (300 MHz); ^{13}C (75 MHz), $CDCl_3$

Table 4. LC_{50} value of methanol extract and *n*-hexane fraction with maceration method

Extract / Fraction	Replication	LC_{50} (ppm)	Mean ± SD (ppm)
Methanol	1	42.05	
	2	41.38	41.40 ± 0.63
	3	40.78	
n-Hexane:EtOAc (10:0)	1	37.05	
	2	38.41	38.16 ± 1.41
	3	36.53	

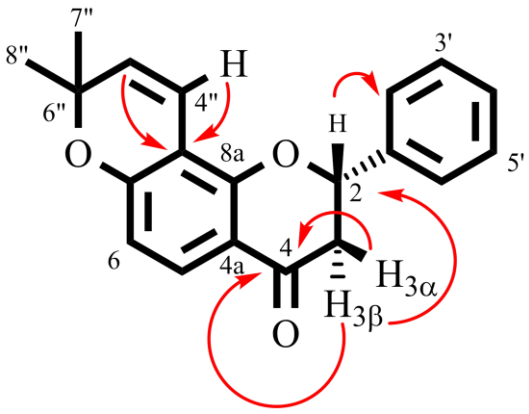

Figure 3. Selected Heteronuclear multiple-bond correlation for isolonchocarpin Arrows point from carbon to proton

The average LC_{50} value obtained through maceration method was 41.40 ppm, with a deviation standard of 0.63. This result indicates the potential toxic effect of *T. vogelii* pod peels methanol extract at below 1,000 ppm (Meyer *et al*., 1982). Based on statistical analysis, the *n*-hexane fraction (fraction I) yielded an LC_{50} of 38.16 at 1.41 ppm deviation standard. Therefore, the results indicate the relatively higher toxicity in fraction I compared to the methanol extract, due to the smaller value. Furthermore, the pure isolonchocarpin subsequently isolated from Fraction I is assumed to also demonstrate toxicity.

4. CONCLUSION

This study highlights valuable data on the cytotoxic effect of *T. vogelii* pod peels. The flavanone investigated and identified from the *n*-hexane fraction was isolonchocarpin, which demonstrated an LC_{50} with greater toxicity than the methanol extract. Hence, this yield is assumed to also possess strong cytotoxic property. However, further evaluation is needed to determine further effects, in order to provide the complete safety profile as a phytotherapeutic agent for generally recommended use.

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